SUPPLEMENTARY METHODS

Peripherally-driven myeloid NFkB and IFN/ISG responses predict malignancy risk, survival, and immunotherapy regime in ovarian cancer

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1. Ovarian cancer mouse model

Six to eight-week-old, female C57BL/6 mice (Envigo, Horst, The Netherlands) were intraperitoneally (i.p.) injected with 5 x 10^6 ovarian cancer ID8 cells. Sample size was determined via statistical power analysis. Eight mice per treatment group had a power of 0.8360 to detect differences between groups. Mice were co-housed in groups of five in individually ventilated cages (IVC) containing wood shaving bedding and nesting materials at the Specific-pathogen-free (SPF) facilities of the KU Leuven. *In vivo* tumour growth was monitored using bioluminescence imaging (BLI). For BLI analyses, mice were scanned one and six week(s) after tumour inoculation. During the scan, mice were anesthetized with isoflurane (2L/min, IsoVet, Dechra Veterinary products n.v, Lille, Belgium). Excess hair of the abdominal and pelvic regions was removed before scanning via mechanical trimming and Veet® hair removal spray. Next, mice were injected subcutaneously with D-luciferine (126 mg/kg, Promega, Madison, US). The total photon flux (in photons per second, p/s) was measured with the IVIS Spectrum Preclinical in vivo Imaging System (PerkinElmer, Waltham, US) using the Living Image Software at the K.U. Leuven core facility (Molecular Small Animal Imaging Center). All animal care takers, except the one responsible for administering therapeutics, were blinded from treatment groups and independently judged the onset of symptoms. Examination of the animals increased from three weekly to once daily if the first signs of disease were detected. Ascites fluid of end-stage mice, reaching 32 grams in overall body weight, was drained by means of puncturing the left abdominal flank. Severely ill mice were sacrificed based on human endpoints as previously published [1]. All experiments performed are in line with the Belgian (Royal Decree, 29 May 2013), Flemish (Decision of the Flemish Government to adapt the Royal Decree of 29 May 2013, 17 February 2017) and European (Directive 2010/63/EU) regulations on the protection of animals used for scientific purposes. Approval of the local ethical committee was obtained (P125/2017) for all mice experiments prior to the start.

2. Therapeutic treatment procedures in mice

Treatment groups were randomly attributed. Both the chemotherapeutic agents and anti-TNF therapy were administered via i.p. injection in the right flank of the abdomen. Chemotherapy was administered two weeks after tumour inoculation and consisted of two separate administrations of carboplatin (100 mg/kg, Carbosin, Teva pharma Belgium n.v., Antwerp, Belgium), and paclitaxel (10 mg/kg, Fresenius Kabi n.v., Schelle, Belgium) as described previously [2]. Animals experiencing chemotherapy toxicities (e.g., weight loss, diarrhea, cachexia and severe hunch back) were sacrificed prematurely and were excluded from data analysis. Anti-TNF (5 mg/kg) was administered biweekly for six weeks. The time schedule for administering the anti-TNF therapy consistent of a clinically-relevant 'late regime' wherein therapy started three weeks after tumour inoculation. The PARP inhibitor, olaparib (50 mg/kg, AZD-2281, MedKoo Biosciences Inc., Morrisville, USA), was administered via oral gavage, once daily for a total of four weeks,

starting at day 21 after tumour inoculation. Control mice received "vehicle" i.p. injections consisting of Dulbecco's phosphate buffered saline (DPBS) (Gibco, Thermo Fisher Scientific, Waltham, US) and oral gavages comprising of a mixture of 10% dimethyl sulfoxide (DMSO) (CryoSure-DMSO, WAK-Chemie Medical GmbH, Steinbach, Germany), 50% Polyethylene glycol 300 (PEG300) and 40% DPBS.

3. Luminex analyses for human and murine serum samples

This analysis was done as described previously [3]. For human serum, the assays were performed with 150 µL of serum. Customized Procartaplex™Immunoassays Kits were purchased (Life Technologies, Merelbeke, Belgium) to determine IL10, IFNα, IL12p70, LAG3, IL6, IDO1, PGF, Arginase, PD-L1, IFNγ, PD1, CXCL10, CCL2, CCL11, CCL22, VEGF-A, TIM3, CCL5, MMP9, and LAP/TGFβ. Herein, 96 well-plates were loaded with antigen-specific capture antibody-coated magnetic beads. Next, samples, standards and blanks were added and incubated for two hours together with the beads. The High Sensitivity IL-10 plex required an overnight incubation. Biotinylated detection antibodies were added as a second step. The antibody/antigen complex was then visualized by incubation with streptavidin-conjugated R-phycoerythrin. In between all steps of the protocol, a washing procedure was performed by means of a magnetic plate washer. Read-out was performed with Bio-plex 200 system of Bio-Rad (Hercules, California, USA). Concentrations of the proteins were determined using five parameter log curves generated by the Bio-Plex Manager 4.1.1 software. Of note, in some cases due to practical (e.g., low quality of samples or below detection threshold data for Bio-plex 200 system), or technical (e.g., not enough sample volumes for profiling) reasons, certain Luminex® data weren't available for every sample. For murine serum, murine blood samples for serum preparation were taken on day -1 (before) and on day +14 and day +34 (after) ID8 cancer cell inoculations. Mice were anesthetized with 1.5% isoflurane in a 70:30 nitrous oxide/oxygen mixture. Glass microhematocrit capillary tubes were inserted in the retro-ocular space and blood was collected from the ophthalmic venous plexus. TNF was measured with Luminex®, according to the manufacturers' protocol using customized Procartaplex™ Immunoassay Kits (Life Technologies, Merelbeke, Belgium) as described above.

4. Murine serum screening via sFIS assay

Whole blood was collected from mice via a submandibular vein puncture at two weeks (i.e., before any *in vivo* treatment regime were commenced; see above) and seven weeks (i.e., after *in vivo* commencement of treatments) after tumour inoculation. During the procedure, mice were anesthetised with isoflurane (2L/min, IsoVet, Dechra Veterinary products n.v, Lille, Belgium). Collected blood was centrifuged at 8000 RCF for ten minutes to obtain serum. Serum was collected and stored at -80°C until further analysis. The murine serum was screened using the J774-Dual™ cells (Invivogen) expressing: (I) the Lucia luciferase

gene, that encodes a secreted luciferase, under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements, and (II) a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an IFN-β minimal promoter fused to 5 copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. These J774 cells were cultured at 37°C under 5% CO2 in DMEM containing 2 mM L-glutamine, 3.7 g/l sodium bicarbonate, 4.5 g/l glucose and 1 mM sodium pyruvate, 100 units/ml penicillin, 100 µg/L streptomycin, 100 µg/ml Normocin and 10% heat inactivated fetal calf serum. For selection of dual reporter expressing cells, the cells were cultured with 5 µg/ml blasticidin and 100 µg/ml zeocin after two passages subsequently to thawing. For mouse serum analysis, J774 were treated with mouse serum for 24 h. Luciferase was checked in media by adding 50 µl of Quanti-Luc (Invivogen) to 100 µl of (separately recovered) J774 media. Bioluminescence was examined for 100 ms immediately after Quanti-Luc addition by microplate reader (Biotek). SEAP activity was checked in media by adding 100 µl of Quanti-Blue (Invivogen) to 100 µl of (separately recovered) J774 media. Absorbance was examined at an optical density of 655 nm, 4 to 8 h after Quanti-Blue addition by microplate reader (Biotek).

5. *In silico* gene signature-based drug or drug-target screening

The in-silico drug-prediction analyses was executed for the NFkB response signature, using the WebGestalt workflow, wherein the "drug" functional database was computed with over-representation analyses (ORA) utilizing a computational algorithm called GLAD4U [4], to screen biomedical literature-associated drug-gene relationships to predict drugs or drug-target's associations to the NFkB response-signature. The following default cut-offs were used: minimum number of genes for a category, 5; maximum number of genes for a category, 2000; multiple adjustment test, Bonferroni; significance level, adjusted p-value=0.01; number of categories expected from set cover, 10.

6. MELODI algorithm-based automated literature screening

We screened the PubMed literature in the time-range of publications from 1950-2017 (on 09/06/2019) via Mining Enriched Literature Objects to Derive Intermediates (MELODI) algorithm [5]. Briefly, the MELODI literature intermediates were retrieved between 'cancer patient serum' search string ((cancer OR tumour OR tumour OR neoplasm) AND (patient OR clinic OR subject) AND (plasma OR serum OR peripheral OR blood)) and 'immunological factors' search string (cytokine OR chemokine OR danger signals OR damage-associated molecular patterns OR DAMPs OR hematopoietic factors OR immune factors). The literature intermediates were retrieved from SemMedDB concepts database [6], a repository of semantic predications (subject–predicate–object triples) extracted from PubMed citations, with the following filtering cut-offs: type of factors (amino acid, peptides, proteins, gene, genomes), mean odds = 5

and p-value = 1.00e-08. This search retrieved the following 54 final hits: CCL17, CCL19, CCL2, CCL20, CCL22, CCL27, CCL3, CCL4, CCL5, CXCL1, CXCL10, CXCL12, CXCL13, CXCL8, CXCL9, CRP, DBP-maf, PF4, THBS1, THPO, VEGFA, G-CSF, FLT3LG, GM-CSF, IFNA1, IFNG, IL10, IL11, IL12p40, IL12p70, IL13, IL15, IL17, IL18, IL1β, IL2, IL21, IL22, IL23, IL3, IL4, IL5, IL6, IL7, IL9, LTA, MCSF, TGFB1, TNF, FASLG, GZMA, GZMB, PRF1 and TRAIL. These 54 hits derived from this MELODI search were annotated for respective cytokine or chemokine families using manual literature-based cross-matching [7, 8].

7. Transcription factor bioinformatics and systems biology/pathway analysis

For computational enrichment analyses enumerating transcription factor (TF)-targets, we used the above 54 hits to enrich for top 3 REACTOME signalling pathways using the WebGestalt workflow. Thereafter, using these REACTOME signalling pathway hits, we enumerated transcription factor targets (TFT) in the C3 gene-set of the Molecular Signatures Database (MSigDB) [9] within Broad Institute's gene-set enrichment analyses (GSEA) portal at statistical significance cut-off of p<1e-25. Finally, based on these TFT hits, we enriched for top 3 Hallmark gene-sets, also within the MSigDB portal. This allowed us to approach transcription factor enrichment in a multi-parametric but integrative manner.

GSEA-based biological pathway enrichment analyses based on multiplexed serum immuno-biomarker concentrations was performed via WebGestalt workflow, using a combination of REACTOME and Wikipathway functional databases with the following selection cut-offs: minimum number of genes for a category, 3; maximum number of genes for a category, 2000; significance level, top 10; number of permutations, 1000; p=1; collapse method, median; number of categories expected from set cover, 10. Redundancy within REACTOME/Wikipathway gene-sets was reduced using affinity propagation method.

Immunological functional network analyses was executed using ImmuNet workflow [10], which creates immune-related functional relationship networks using pathway-relevant gene-gene relationship predictions based on 38,000 genome-scale experiments. For TGFB1 network with IFN response, we used STAT1, TGFB1, IFIT2, IRF9 and STAT2, while for MMP9/HAVCR2 network with NFkB response, we used REL, NFKB1, MMP9, RELA, HAVCR2 to initialize networks within "Immune Global" pathway. Following network filters were used: minimum relationship confidence of 0.2, and maximum number of genes at 20. Herein, biological process enrichment analyses were used to delineate genes/proteins connecting relevant components at statistically significant threshold of p<0.05.

8. NFkB or IFN response genetic signatures for bioinformatics analysis

To use experimentally pre-validated genetic signatures for NFkB signalling pathway as well as general IFN response pathways, we started with existing GSEA gene-sets within the C2: curated gene sets with CGP:

chemical and genetic perturbations subsets. For NFkB response signature, we selected the TIAN_TNF_SIGNALING_VIA_NFKB gene-set (systematic name: M14435) consisting of the following genes: CCL20, CCN2, CD83, CXCL1, CXCL2, CXCL3, CXCL8, GCH1, GFPT2, IL6, IRF1, NFKB1, NFKB2, NFKBIA, NFKBIE, PLK2, PTGS2, REL, RELB, SDC4, TNFAIP2, TNFAIP3, TNFRSF9, TNIP1, TRAF1, ZFP36. For IFN response signature, since there was a paucity of a clear gene-set simultaneously applicable to all three major IFN cytokines i.e., IFNα, IFNβ and IFNγ, we decided to construct a consensus gene signature ourselves. Herein, we extracted the following gene sets from the above database (GSEA: C2-CGP): SANA_RESPONSE_TO_IFNG_UP (systematic name: M4551), MOSERLE_IFNA_RESPONSE (systematic name: M3218), and HECKER_IFNB1_TARGETS (systematic name: M3010), and created a signature with following genes that were overlapping across all of these three datasets thereby making this a broadly IFN response-applicable signature: CXCL10, GBP1, MX1, IFI44L, OAS2, SAMD9L, DDX60, IFIH1, LGALS3BP, STAT1, TRIM22, IFI35, TNFSF10, DTX3L, PARP9, SERPING1, LAP3, CD274, MMP25. Next, we wanted to make sure that these genetic signatures do not suffer from dataset/gene-set selection-bias, or bias due to experimental/perturbation contexts from which they were originally derived. To this end, we used these signatures to run a MSigDB database gene-overlap analyses within the C2-CGP gene-sets. This analyses delineated following statistically significantly overlapping top 10 gene-sets for the NFkB response signature: TIAN_TNF_SIGNALING_VIA_NFKB (p-value = 2.44e-95; FDR q-value = 8.06e-92), PHONG_TNF_TARGETS_UP (p-value = 1.02e-40; FDR q-value = 1.69e-37), ZHANG_RESPONSE_TO_IKK_INHIBITOR_AND_TN_TNF_UP (p-value = 3.14e-39; FDR q-value = 3.46e-36), HINATA_NFKB_TARGETS_FIBROBLAST_UP (p-value = 8.07e-33; FDR q-value = 6.67e-30), ZHOU_INFLAMMATORY_RESPONSE_LIVE_UP (p-value = 5.32e-31; FDR q-value = 3.51e-28), LINDSTEDT_DENDRITIC_CELL_MATURATION_B (p-value = 4.77e-30; FDR q-value = 2.62e-27), ZHOU_INFLAMMATORY_RESPONSE_FIMA_UP (p-value = 6.71e-30; FDR q-value = 3.16e-27), HINATA_NFKB_TARGETS_KERATINOCYTE_UP (p-value = 5.78e-27; FDR q-value = 2.39e-24), PHONG_TNF_RESPONSE_NOT_VIA_P38 (p-value = 1.57e-21; FDR q-value = 5.75e-19), and PHONG_TNF_RESPONSE_VIA_P38_PARTIAL (p-value = 6.27e-21; FDR q-value = 2.07e-18). Similarly, above analyses delineated following statistically significantly overlapping top 10 gene-sets for the IFN response signature: SANA_RESPONSE_TO_IFNG_UP (p-value = 5.56e-241; FDR q-value = 1.84e-237), SANA_TNF_SIGNALING_UP (p-value = 1.57e-67; FDR q-value = 2.59e-64), BROWNE_INTERFERON_RESPONSIVE_GENES (p-value = 1.85e-33; FDR q-value = 1.76 e-30), TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_3N_3D_UP (p-value = 2.14e-33; FDR q-value = 1.76 e-30), WIELAND_UP_BY_HBV_INFECTION (p-value = 3.65e-32; FDR q-value = 2.41 e-29), MOSERLE_IFNA_RESPONSE (p-value = 3.9e-29; FDR q-value = 2.15e-26), HECKER_IFNB1_TARGETS (p-value = 1.89e-28; FDR q-value = 8.26e-26), NUYTTEN_EZH2_TARGETS_UP (p-value = 2e-28; FDR

q-value = 8.26e-26), GAURNIER_PSMD4_TARGETS (p-value = 4.7e-28; FDR q-value = 1.72e-25), and RADAEVA_RESPONSE_TO_IFNA1_UP (p-value = 9.4 e-24; FDR q-value = 3.1e-21). These analyses established that above NFkB or IFN response signatures can capture variety of biological and immunological contexts associated with these pathways.

9. Genetic-signature overlap analyses for IFN-cytokine subtypes

To understand the overall overlap or sharing between downstream IFN-induced genes between type I IFN-cytokines, type II IFN-cytokines and type III IFN-cytokines for our consensus ISG-signature, as well as separate signatures induced downstream of IFNα, IFNβ or IFNγ (see above), we used the "IFN Subtype" computational tool within the Interferome v2.0 database. Interferome v2.0 is an online resource (http://interferome.its.monash.edu.au/interferome/) that integrates computational analyses based on curated experimental datasets on types I, II and III IFN-treated human or mice cells. The "IFN Subtype" tool produces Venn diagrams representing how many genes are regulated by each IFN cytokine-type and allows estimation of the overlaps of such genes regulated by two or all three IFN cytokine-types.

10. Transcriptomic analysis with The Cancer Genome Atlas (TCGA) datasets

a. Gene-expression analyses: For these analyses we wanted to randomly select 6 immunotherapy-responsive and 6 immunotherapy-resistant TCGA cancer-types for further analyses (with 6 being the minimum number of variables required for a trustworthy correlation analyses). Hence, we took all the highly immunotherapy-responsive as well as medium-to-low immunotherapy-responsive cancer-types from an existing publication [11] and applied a "List Randomizer" computational program (https://www.random.org/lists/) to randomly select at least 6 cancer-types across both categories. These analyses delineated the following 12 cancer-types: Liver cancer, Pancreatic cancer, Lung cancer, Head and Neck cancer, Cervical cancer, Bladder cancer, Endometrial cancer, Ovarian cancer, Sarcoma, Breast cancer, or Kidney cancer/RCC. Expression analyses for 54 MELODI hits was performed for 12 different TCGA cancer-cohorts using the GEPIA2 workflow [12]. These included, Liver hepatocellular carcinoma (LIHC, n=369), Pancreatic adenocarcinoma (PAAD, n=179), Lung squamous cell carcinoma (LUSC, n=486), Lung adenocarcinoma (LUAD, n=483), Head and Neck squamous cell carcinoma (HNSC, n=519), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, n=306), Bladder Urothelial Carcinoma (BLCA, n=404), Uterine Corpus Endometrial Carcinoma (UCEC, n=174), Ovarian serous cystadenocarcinoma (OV, n=426), Sarcoma (SARC, n=262), Breast invasive carcinoma (BRCA, n=1085), or Kidney renal clear cell carcinoma (KIRC, n=523). Median expression value [log (TPM + 1) transformed expression] of each gene was normalized by the maximum median expression value across all genes.

b. Survival analysis: For overall survival (OS) estimation, tumour bulk-RNA-seq HTSeq counts, and patient OS data were accessed for 12 different TCGA cancer-cohorts using the Kaplan-Meier Plotter's Pan-cancer RNA-seq workflow [13]. These included, Liver hepatocellular carcinoma (LIHC, n=371), Pancreatic adenocarcinoma (PAAD, n=177), Lung squamous cell carcinoma (LUSC, n=501), Lung adenocarcinoma (LUAD, n=513), Head and Neck squamous cell carcinoma (HNSC, n=500), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, n=304), Bladder Urothelial Carcinoma (BLCA, n=405), Uterine Corpus Endometrial Carcinoma (UCEC, n=543), Ovarian serous cystadenocarcinoma (OV, n=374), Sarcoma (SARC, n=259), Breast invasive carcinoma (BRCA, n=1090), or Kidney renal clear cell carcinoma (KIRC, n=530). The survival plots were based on auto-selected best cut-off thresholds for genetic expression (i.e., all possible cut-offs are calculated between lower/upper quartile and best performing threshold is used), with all follow-up thresholds used, while censoring at threshold and median computed over the entire database, with estimations for hazard ratio (HR) as well as log-rank p-value (threshold <0.05). NFkB or IFN response signatures were computed based on mean expression of genes. Patients belonging to all stages, genders, race, grade, and mutational burden were included without exclusion.

11. GEO dataset analyses: Gene expression data (Affymetrix GeneChip Human Genome U133Plus 2.0 arrays) for (bulk) ascites cells from 9 OV patients (pre and during treatment with infliximab) were derived from a previously published resource (GSE18681) [14]. These data were processed through a unified ShinyGEO workflow (https://gdancik.github.io/shinyGEO/) [15]. All values were $\log_2$ transformed.

12. Analysis of cancer patient's single-cell RNA-sequencing (scRNAseq) datasets

For single-cell expression analyses we wanted to analyse two types of datasets i.e., a scRNAseq dataset with immune cells' single-cell profiling in both blood as well as tumours in a patient-matched fashion (with very high-number of single-cells profiled), and a scRNAseq dataset with pan-ovarian tumour single-cell profiling including both cancer cells as well as immune cells (with very high-number of single-cells profiled across as many patients as possible). At the time when the data-driven framework for this study was being constructed, only one scRNAseq dataset was available that profiled high number of single immune cells from both blood and tumour in a patient-matched fashion i.e., GSE139555 dataset [16]. On the other hand, for ovarian tumour, at least 4 different pan-tumour datasets were available, with scRNAseq data for 1 patient [17], 2 patients [18], 5 patients [19] and 7 patients [20]. Herein, we wanted to use the scRNAseq dataset with highest number of patients as well as high number of single-cells profiled however, the dataset with 7 patients profiled only 2911 single-cells, while the dataset with 5 patients profiled a very high amount i.e., 45114 single-cells. Owing to a sufficient number of patients and high number of single-cells we decided

to continue with the latter dataset. Single-cell expression data (log-normalized from raw expression counts) for the following existing human patient datasets: (I) tumour-infiltrating (n=93303 cells) or blood-associated (n=25550 cells) immune cells, consisting of myeloid cells (n=29866), CD4+T cell (n=12100), B cell (n=9769), T cell (n=2125), effector memory CD8+T cell (n=3279), tissue-resident memory CD8+T cell (n=8299), plasma cell (n=404), NK cell (n=3363), CD8+T cell (n=1304), tissue-resident memory CD4+T cell (n=2344), or Treg (n=1681), from renal cell (n=3 patients) or large cell neuroendocrine carcinoma patients (n=1 patient) (derived from GSE139555 dataset) [16], and (II) cancer cells (n=14134 cells) or tumour-infiltrating immune cells like B cells (n=964 cells), myeloid cells (n=7260 cells), endothelial cells (n=1891 cells), fibroblasts (n=14760 cells), T/NK cells (n=6105 cells) from ovarian cancer patients (n=5 patients) (10x scRNAseq data derived from http://blueprint.lambrechtslab.org/) [19], were accessed as well as processed using a standardized and uniformized workflow of BioTuring's BBrowser 2 [21]. Data was derived for single-cells that passed the default built-in qualitative filters for minimum cells per gene, minimum genes per cell, maximum mitochondrial fraction per cell and number of highly variable genes. In-build BioTuring – Cell type or original study's labels were utilised, depending on whichever labels were most broad [21]. The dimensionality reduction was achieved using a two-dimensional t-distributed stochastic neighbour embedding (t-SNE) space, available within the BBrowser 2 [21]. The input parameters for these t-SNEs were, perplexity=30 and initial_dims=30. Genetic signatures were created based on above NFkB response or ISG response genetic signatures (by adding the total gene expression values) and plotted onto each single-cell within above t-SNEs to create a signalling signature scRNAseq map. Data was also represented as violin plots (i.e., overall NFkB or ISG signature expression levels per immune cell-type) or correlation analyses (i.e., Spearman correlation analyses between NFkB and ISG signature expression in the myeloid cells) as applicable. In case of the OV scRNAseq dataset, dot plot was created for type I (*IFNA1, IFNB1, IFNA2, IFNE*) or type II (*IFNG*) IFN-cytokine genes (gene expression representation for portion of total cells per subtype, while including zero expression values). Finally, for differential pathway enrichment analyses the DESeq2 function in the BBrowser 2 [21] pipeline was utilized. Briefly, in the OV scRNAseq dataset, NFkB or ISG signature's expression distribution was plotted across all the myeloid cells and they were stratified into NFkB signature-response^{HIGH} or ISG signature-response^{HIGH} subgroups on the basis of median expression cut-off for both signature distributions. Differential gene expression analyses was carried out between these two subgroups of the myeloid cells, coupled with differential REACTOME biological pathway enrichment analyses via the DESeq2 module.

13. Statistical analyses and data visualization

The statistical details of all the analyses are reported either in the figure legends, figures and/or methods sections, including statistical analysis performed, statistical significance thresholds/values and in most cases the counts/number of data-points. All the statistical tests used herein were always two-tailed unless otherwise explicitly mentioned. Gene signatures were estimated by considering the average expression of all the genes within that signature, unless otherwise mentioned.

Within the discovery cohort, multi-variate survival analysis prediction was calculated using the lifelines module for python 3.8.6. The regression models were built using age, NFkB response, IFN response, and tumour stage co-variates for PFS and OS. These analyses were used to estimate CoxPH coefficient and the lower and upper 95% intervals. Survival probability curves (Kaplan Meier) were plotted for the baseline and for a fixed value co-variate estimated curves (NFkB response and IFN response). All statistical analyses, correlation matrices, heatmaps or graphical representations were executed using Python version 3.7.3, ClustVis (probabilistic PCA based on vector row scaling) [22], Morpheus (https://software.broadinstitute.org/morpheus/), or GraphPad Prism version 8/9, as feasible or applicable.

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